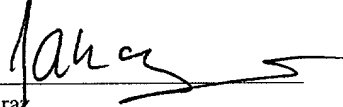


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Tamara Alcaraz

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In the application of:

Jerry PELLETIER

Serial No.: To Be Assigned

Filing Date: Herewith

For: METHOD FOR INCREASING THE  
PROCESSIVITY OF A DNA- OR RNA-  
DEPENDENT POLYMERASE AND  
COMPOSITIONS THEREFOR

Examiner: To Be Assigned

Group Art Unit: To Be Assigned

**PRELIMINARY AMENDMENT**

Box PATENT APPLICATION  
Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

Prior to examination of the above-captioned application, please enter the following amendments.

**AMENDMENTS**

**In the Specification:**

*Please replace the following paragraphs starting on page 18, line 5 and ending on page 20, line 23*

Fig. 1 (PRIOR ART) shows an example of the steps involved in generating cDNA libraries from mRNA. Although a number of strategies can be used for cDNA library generation, of which two are shown above, all libraries require as a first step, a primer from which the reverse transcriptase (RT) can prime. In the case of full-length cDNA libraries, an oligo d(T) primer is used because it anneals to the 3' poly (A) tail of the eukaryotic mRNAs. In the case of prokaryotic, some viral, or other eukaryotic

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mRNAs which lack a poly (A) tail, a homopolymeric stretch of nucleoside 5'-monophosphates can be added to the 3' end of the mRNA. For example, poly (A) polymerase can be used to add a poly (A) tail to mRNAs which lack one. An oligonucleotide which contains complementary nucleotides (e.g. oligo d(T)) is then annealed to the mRNA and serves as primer for the RT;

Fig. 2 shows a schematic diagram illustrating the test constructs generated, in which stable stem-loop structures were inserted into the Nco I site of the WT1 gene. A Sau 3AI fragment of the WT1 gene was inserted into pSP65(T), positioning a tract of 38 adenosine residues downstream of the WT1 gene, allows first strand synthesis to be primed by an oligo d(T) primer. Clones containing either one or two copies of the (M1/X) stem-loop structures were isolated and characterized by sequencing. Insertion of one or two copies of GNRA stem-loop structure was done in flWT1, a derivative of pSP/WT1 in which a portion of the GC-rich 5' UTR of WT1 was present. Termination of RT by the stem-loop structures is expected to generate a truncated product of ~920 bases, whereas full-length copying of the template is expected to produce a product of ~1.4 - 1.5 kb in the case of pSP/WT1(M1/X) and a product of ~1.9 - 2.0 kb in the case of pSP/flWT1(GNRA).

Figs. 3A and 3B show an assessment of the denaturation conditions known in the art to improve RT processivity during first strand cDNA synthesis. Fig. 3A First strand RT reactions were performed with oligo d(T) and Superscript II utilizing either RNA made from in vitro transcription of pSP/WT1 (lane 1), pSP/flWT1 (lane 2), or pSP/flWT1(GNRA)2 (lanes 3-11). Inhibition of RT processivity by the GNRA stem-loop structure is expected to yield a product of ~920 bases (denoted by an asterisk). Addition of oligo d(T) primer before the RT enzyme (lanes 1-3, 5-11) or addition of oligo d(T) primer after the RT enzyme (lane 4), did not affect the efficiency of first strand synthesis (compare lane 4 to lane 3). Prior denaturation of the RNA before the RT reaction did not improve the processivity of the RT (lanes 5 - 11). Denaturation at 65°C / 5 min (lane 5), denaturation at 65°C for 5 min, followed by snap freezing on dry ice and slow thawing on ice (lane 6), denaturation with methylmercury hydroxide (lane 7), denaturation in 5% DMSO (lane 8), denaturation in 5% DMSO/35% glycerol (lane 9), or performing the RT in the presence of Trehalose at 45°C (lane 10) or at 55°C (lane 11) did not improve the processivity of Superscript II as judged by the presence of the ~ 920 base truncated cDNA product in all the lanes. Fig. 3B Pre-incubation of either flWT1 or flWT1(GNRA)1 with eIF-4A and/or eIF-4B does not result in improvement of Superscript II processivity. The nature of the RNA template in the RT reaction is outlined to the left and the addition of eIF-4A (an RNA helicase capable of unwinding RNA secondary structure) and/or eIF-4B (an RNA binding protein that works in conjunction with eIF-4A) is outlined to the right of the figure. The position of migration of the truncated cDNA products is indicated by asterisks.

Fig. 4A (SEQ ID NO:1) shows primary amino acid structure of HIV NCp7, a well characterized nucleic acid chaperone protein. The two zinc fingers are underlined. [B)] Fig. 4B shows titration of NCp7 on RT reactions performed with Superscript II (lanes 1 - 10) and AMV RT (lanes 11 -12). The nature of the input RNA is shown below the panel. Asterisks denote the major truncated RT products obtained due to termination of DNA synthesis by the RT enzyme when regions of secondary structure are encountered by the enzyme.

Fig. 5A shows a general scheme to assess the effect of general DNA binding proteins on the processivity of DNA polymerases. First strand cDNA product is generated from *in vitro* transcribed WT1 mRNA. The RNA moiety of this product is hydrolyzed under alkaline conditions (50 mM NaOH/60°C/30 min.), and the remaining ssDNA is tailed at the 3' end with terminal deoxynucleotidyl transferase and dTTP. General DNA binding proteins are assessed in the presence of T7 DNA polymerase, an oligo d(A) primer, and radiolabelled  $\alpha$ -<sup>32</sup>P-dATP. After termination of the reactions, the products are fractionated on a alkaline 1% agarose gel and visualized by autoradiography. Fig. 5B Addition of DNA binding proteins to T7 DNA polymerase during second strand synthesis improves yield of product. Second strand reactions were supplemented with nothing (lane 1), 2  $\mu$ g T4gp32 (lane 2), 2  $\mu$ g SSB (lane 3), or 2  $\mu$ g rec A (lane 4). The arrow denotes the position of migration of full-length second strand product whereas the asterisk denotes the position of migration of truncated product.

#### In the Claims:

Please cancel claims 10, 11, 29, and 30.

Please amend the following claims.

4. (Amended) The method of claim 1, wherein said RNA binding protein is a retroviral nucleocapsid protein.

8. (Amended) The improved method of claim 6, wherein said RNA binding protein is a retroviral nucleocapsid protein.

14. (Amended) The method of claim 12, wherein said polymerase is MMTV RT or AMV RT.



## REMARKS

Claims 1-9, 12-28, and 31 are pending. Claims 4, 8, 14, 17, 20, 24 and 27 have been amended. Claims 10, 11, 29 and 30 have been cancelled. The amendments to the claims have been made to remove multiple dependency and reduce filing fees. These amendments and cancellation of claims are not intended to abandon, disclaim or dedicate any subject matter.

The above amendments to the specification have been made to correct typographical and grammatical errors. Accordingly, Applicants submit no new matter by these amendments.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

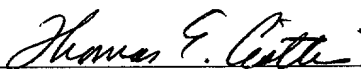
In the unlikely event that the Patent Office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 03-1952 referencing docket no. 514012000300.

*However, the Assistant Commissioner is NOT authorized to charge the cost of the issue fee, extra claim fees or filing fees to the Deposit Account. Applicant wishes to pay extra claim and filing fees with Response to Missing Parts of Application.*

Respectfully submitted,

Dated: September 12, 2001

By:



Thomas E. Ciotti  
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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**In the Specification:**

*Please replace the following paragraphs starting on page 18, line 5 and ending on page 20, line 23*

[Figure 1] Fig. 1 (PRIOR ART) shows an example of the steps involved in generating cDNA libraries from mRNA. Although a number of strategies can be used for cDNA library generation, of which two are shown above, all libraries require as a first step, a primer from which the reverse transcriptase (RT) can prime. In the case of full-length cDNA libraries, an oligo d(T) primer is used because it anneals to the 3' poly (A) tail of the eukaryotic mRNAs. In the case of prokaryotic, some viral, or other eukaryotic mRNAs which lack a poly (A) tail, a homopolymeric stretch of nucleoside 5'-monophosphates can be added to the 3' end of the mRNA. For example, poly (A) polymerase can be used to add a poly (A) tail to mRNAs which lack one. An oligonucleotide which contains complementary nucleotides (e.g. oligo d(T)) is then annealed to the mRNA and serves as primer for the RT;

[Figure 2] Fig. 2 shows a schematic diagram illustrating the test constructs generated, in which stable stem-loop structures were inserted into the Nco I site of the WT1 gene. A Sau 3AI fragment of the WT1 gene was inserted into pSP65(T), positioning a tract of 38 adenosine residues downstream of the WT1 gene, allows first strand synthesis to be primed by an oligo d(T) primer. Clones containing either one or two copies of the (M1/X) stem-loop structures were isolated and characterized by sequencing. Insertion of one or two copies of GNRA stem-loop structure was done in flWT1, a derivative of pSP/WT1 in which a portion of the GC-rich 5' UTR of WT1 was present. Termination of RT by the stem-loop structures is expected to generate a truncated product of ~920 bases, whereas full-length copying of the template is expected to produce a product of ~1.4 - 1.5 kb in the case of pSP/WT1(M1/X) and a product of ~1.9 - 2.0 kb in the case of pSP/flWT1(GNRA).

[Figure 3 shows] Figs. 3A and 3B show an assessment of the denaturation conditions known in the art to improve RT processivity during first strand cDNA synthesis. [A] Fig. 3A First strand RT reactions were performed with oligo d(T) and Superscript II utilizing either RNA made from in vitro transcription of pSP/WT1 (lane 1), pSP/flWT1 (lane 2), or pSP/flWT1(GNRA)2 (lanes 3-11). Inhibition of RT processivity by the GNRA stem-loop structure is expected to yield a product of ~920 bases (denoted by an asterisk). Addition of oligo d(T) primer before the RT enzyme (lanes 1-3, 5-11) or addition of oligo d(T) primer after the RT enzyme (lane 4), did not affect the efficiency of first strand synthesis (compare lane 4 to lane 3). Prior denaturation of the RNA before the RT reaction did not improve the processivity of the RT (lanes 5 - 11). Denaturation at 65°C / 5 min (lane 5), denaturation at 65°C for 5 min, followed by snap freezing on dry ice and slow thawing on ice (lane 6), denaturation with methylmercury hydroxide (lane 7), denaturation in 5% DMSO (lane 8), denaturation in 5% DMSO/35% glycerol (lane 9), or performing the

RT in the presence of Trehalose at 45°C (lane 10) or at 55°C (lane 11) did not improve the processivity of Superscript II as judged by the presence of the ~ 920 base truncated cDNA product in all the lanes. [B)] Fig. 3B Pre-incubation of either f1WT1 or f1WT1(GNRA)1 with eIF-4A and/or eIF-4B does not result in improvement of Superscript II processivity. The nature of the RNA template in the RT reaction is [outlined] outlined to the left and the addition of eIF-4A (an RNA helicase capable of unwinding RNA secondary structure) and/or eIF-4B (an RNA binding protein that works in conjunction with eIF-4A) is outlined to the right of the figure. The position of migration of the truncated cDNA products is indicated by asterisks.

[Figure 4 shows: A) Primary] Fig. 4A (SEQ ID NO:1) shows primary amino acid structure of HIV NCp7, a well characterized nucleic acid chaperone protein. The two zinc fingers are underlined. [B)] Fig. 4B [Titration] shows titration of NCp7 on RT reactions performed with Superscript II (lanes 1 - 10) and AMV RT (lanes 11 -12). The nature of the input RNA is shown below the panel. Asterisks denote the major truncated RT products obtained due to termination of DNA synthesis by the RT enzyme when regions of secondary structure are encountered by the enzyme.

[Figure 5 shows: A) A] Fig. 5A shows a general scheme to assess the effect of general DNA binding proteins on the processivity of DNA polymerases. First strand cDNA product is generated from *in vitro* transcribed WT1 mRNA. The RNA moiety of this product is hydrolyzed under alkaline conditions (50 mM NaOH/60°C/30 min.), and the remaining ssDNA is tailed at the 3' end with terminal deoxynucleotidyl transferase and dTTP. General DNA binding proteins are assessed in the presence of T7 DNA polymerase, an oligo d(A) primer, and radiolabelled  $\alpha$ -<sup>32</sup>P-dATP. After termination of the reactions, the products are fractionated on a alkaline 1% agarose gel and visualized by autoradiography. [B)] Fig. 5B Addition of DNA binding proteins to T7 DNA polymerase during second strand synthesis improves yield of product. Second strand reactions were supplemented with nothing (lane 1), 2 µg T4gp32 (lane 2), 2 µg SSB (lane 3), or 2 µg rec A (lane 4). The arrow denotes the position of migration of full-length second strand product whereas the asterisk denotes the position of migration of truncated product.

#### **In the Claims:**

4. (Amended) The method of claim 1,[ 2, or 3,] wherein said RNA binding protein is a retroviral nucleocapsid protein.

8. (Amended) The improved method of claim 6,[ or 7,] wherein said RNA binding protein is a retroviral nucleocacid protein.

14. (Amended) The method of claim 12[ or 13], wherein said polymerase is MMTV RT or AMV RT.

17. (Amended) The method of claim 15[ or 16], wherein said polymerase is MMTV RT or AMV RT.

20. (Amended) The composition of claim 18[ or 19], wherein said RNA binding protein is the chaperone protein Ncp7.

24. (Amended) The method of claims 22[ or 23], wherein said DNA binding protein is selected from the group consisting of T4gp32, SSB, and rec A.

27. (Amended) The improved method of claim 25[ or 26], wherein said DNA binding protein is a single-strand DNA binding protein.